Diversity studies of some *Phaseolus* taxa using chloroplast DNA as a molecular marker

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INTRODUCTION

Within the genus *Phaseolus*, the taxonomy and phylogeny of *P. coccineus*, *P. polyanthus* and related wild species are poorly understood. *P. polyanthus* has been considered either as a subspecies of *P. coccineus* (MARECHAL *et al.*, 1978; DELGADO, 1985) or as a distinct species of the genus (SMARTT, 1973). Morphological observations as well as results from interspecific hybridizations and recent biosystematic studies favour the attribution of a specific rank to *P. polyanthus* (SCHMIT & DEBOUCK, 1991). In Costa Rica, a wild form related to *P. coccineus* was recently collected and named *P. costaricensis* by DEBOUCK *et al.*[1989]. These authors noted that natural crosses occur between that form and populations of *P. polyanthus* growing near the collecting site. The position of this new form within the *P. coccineus* complex is still unclear. Another taxon of the complex, *P. glabellus*, ecologically and morphologically distinct and so far considered as a subspecies of *P. coccineus*, presents biochemical differences when compared to other taxa of this species. Distinct banding patterns are revealed for this form in seed protein electrophoresis (SCHMIT & DEBOUCK, 1990; SCHMIT *et al.*, 1991). On the other hand, no natural crosses between this form and wild *P. coccineus* populations growing in the same environment have been observed when collecting *P. glabellus* accessions (DEBOUCK, 1987).In our study of these predominantly allogamous taxa, we analysed chloroplast DNA because of the relatively small size and maternal inheritance of this genome.

MATERIALS and METHODS

The 33 *Phaseolus* populations studied include seven taxa belonging to the group *P. vulgaris* - *P. polyanthus* - *P. coccineus* and one population of *P. lunatus* selected for its remoteness from this group. They are listed in table 1. This material comes from the *Phaseolus* collection of Gembloux and from the Genetic Resources Unit of the "Centro Internacional de Agricultura Tropical" (C.I.A.T., Cali, Colombia).The chloroplast DNA was purified using the method of HOSAKA (1986) with minor modifications. The restriction enzymes used were Eco RI, Bam HI, Ava I, Xho I, Eco RV and Hind III. Digestions were performed according to the supplier (Bethesda Research Laboratories). The cpDNA fragments were separated by electrophoresis at 25 mA for 16-20 h in an agarose slab gel containing 40 mM sodium acetate and 1 mM EDTA. Gels were stained with ethidium bromide solution and photographed under long wave U.V. light.

Table 1. *Phaseolus* material used in cpDNA study

Taxon	Number	Country of origin	Biological status	Taxon	Number	Country of origin	Biological status
P. lunatus	NI 1259	Peru	wild	*P. polyanthus*	DGD1631	Guatemala	wild
P. vulgaris	NI 637	Brazil	cultivated		G35771	Guatemala	cultivated
	G 6040	Guatemala	cultivated		G35780	Guatemala	cultivated
	DGD2423	Guatemala	wild		G35755	Guatemala	cultivated
	DGD1616	Guatemala	wild		G35061	Mexique	cultivated
	NI1193	Mexico	wild		G35122	Mexique	cultivated
	DGD2097	Costa Rica	wild		G35337	Mexique	cultivated
	DGD2769	Ecuador	wild		G35380	Mexique	cultivated
	DGD2484	Bolivia	wild		DGD2653	Colombia	escaped
	DGD1716	Argentina	wild		G35314	Colombia	escaped
P. costaricensis	DGD2132	Costa Rica	wild		G35360	Colombia	escaped
Natural hybrids					G35383	Colombia	escaped
between					G35625	Colombia	cultivated
*P.pol.*and *P. vulg.*	DGD2988	Colombia	cultivated	*P.coccineus*			
	DGD2975	Colombia	cultivated	subsp.*coccineus*	NI 755	Guatemala	cultivated
P. pol. and *P.*	G35841	Colombia	cultivated		NI 1204	Mexico	wild
cocc.	NI 1270	Mexico	wild		NI 813	Mexico	wild
P. glabellus				subsp.*obvallatus*	NI 1108	Mexico	wild
				subsp *purpuracens*	NI 722	Mexico	wild

RESULTS and DISCUSSION

Restriction patterns obtained with Hind III are identical for all the populations studied. The other five enzymes enabled us to isolate two taxa from the other ones : those are *P. lunatus*, genetically located at the opposite site from *P. vulgaris* in the genus, and *P. glabellus*.

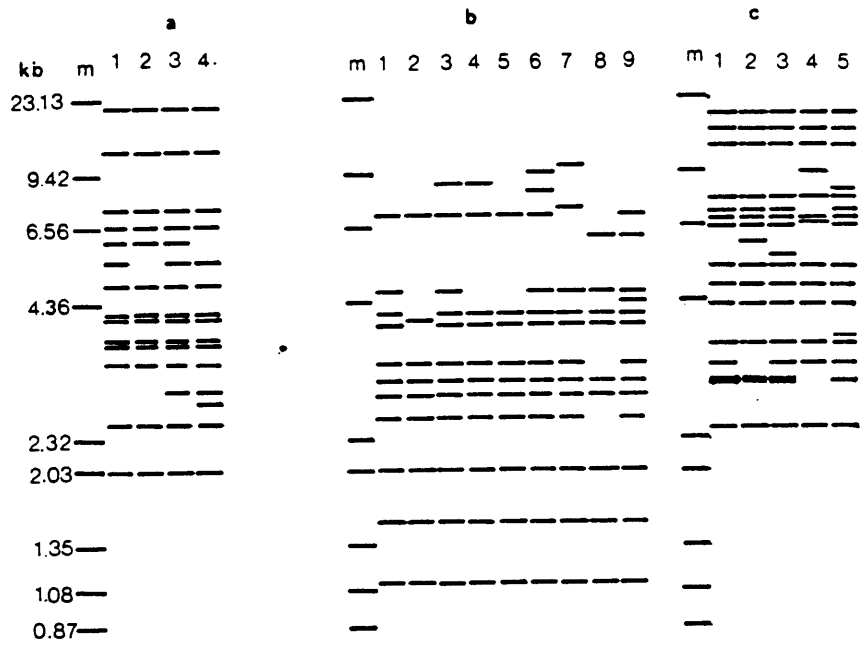

Fig.1. m = marker; a = restriction patterns obtained from digestions by Ava I of : 1 = all populations but NI 755, NI 1108, NI 813, NI 1204, NI 722, G 35841, NI 1270 and NI 1259; 2 = NI 755, NI 1108, NI 813, NI 1204 and NI 722; 3 = NI 1270; 4 = NI 1259; b = restriction patterns obtained from digestions by Eco RI of : 1 = DGD 2988, DGD 2975, all populations of *P. vulgaris* but DGD 2423 and DGD 2769, all populations of *P. polyanthus* but G 35383, G 35314, DGD 1631, G 35771 and G 35780; 2 = NI 813; 3 = DGD 2423, G 35383, G 35314, DGD 2132; 4 = NI 755, NI 1108; 5 = NI 1204, NI 722, G 35841; 6 = DGD 1631, DGD 2769; 7 = G 35771, G 35780; 8 = NI 1270; 9 = NI 1259; c = restriction patterns obtained from digestions by Bam HI of : 1 = all populations but NI 813, NI 755, NI 1108, NI 1270 and NI 1259; 2 = NI 813; 3 = NI 755 and NI 1108; 4 = NI 1270; 5 = NI 1259.

Only three enzymes revealed variability at the cpDNA level for the six remaining taxa. Results are presented in figure 1. Based on restriction patterns obtained with Ava I (fig.1a) and Eco RI (fig.1b), two groups of taxa can be separated, *P. vulgaris*, *P. costaricensis* and *P. polyanthus* on one hand, and the three subspecies of *P. coccineus* on the other hand. Indeed, compared with the patterns obtained from the taxa of the first group, one fragment is missing in Ava I and Eco RI patterns for all the subspecies of *P. coccineus*. This fragment weights about 4,8 kb and 5,2 kb for Eco RI and Ava I patterns respectively. Other variations have been observed for Eco RI patterns within and between taxa of both groups. Either one or multiple fragment(s) is(are) identified between 10 kb and 6,56 kb. This variability should now be investigated further to better understand the phyletic distance among the tested populations. Further cpDNA studies based on inter- and intra-populations variability within the taxa considered will help to achieve this goal.

Patterns obtained with Bam HI showed polymorphism only between some populations of *P. coccineus*, reflecting the high level of diversity in this species. As presented in figure 1c, the wild form NI 813 (lane 2)shows the highest level of polymorphism compared to the patterns of the other populations. Two populations produce an additional fragment of about 5,6 kb (lane 3): NI 755, a cultivated *P. coccineus* and NI 1108, a population belonging to the subspecies *obvallatus*.

These results backen up the position of attributing a specific rank to *P. polyanthus*, as already suggested by results from interspecific hybridizations, morphological observations and biosystematic studies. They also confirm the location of the wild species *P. costaricensis* as an intermediate taxon between *P. vulgaris* and *P. polyanthus*. Finally, *P. glabellus* should be considered as a very distinct species, well separated from the other taxa of the group.

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